OPEN

Equilibrative Nucleoside Transporters 1 and 4: Which One Is a Better Target for Cardioprotection Against Ischemia–Reperfusion Injury?

Cui Yang, PhD and George P. H. Leung, PhD†*

(See editorial: Kuala Lumpur Emerging in Vascular Biology by Paul M. Vanhoutte. Journal of Cardiovascular Pharmacology, 2015 65:4;297–298)

Abstract: The cardioprotective effects of adenosine and adenosine receptor agonists have been studied extensively. However, their therapeutic outcomes in ischemic heart disease are limited by systemic side effects such as hypotension, bradycardia, and sedation. Equilibrative nucleoside transporter (ENT) inhibitors may be an alternative. By reducing the uptake of extracellular adenosine, ENT1 inhibitors potentiate the cardioprotective effect of endogenous adenosine. They have fewer systemic side effects because they selectively increase the extracellular adenosine levels in ischemic tissues undergoing accelerated adenosine formation. Nonetheless, long-term inhibition of ENT1 may adversely affect tissues that have low capacity for de novo nucleotide biosynthesis. ENT1 inhibitors may also affect the cellular transport, and hence the efficacy, of anticancer and antiviral nucleoside analogs used in chemotherapy. It has been proposed that ENT4 may also contribute to the regulation of extracellular adenosine in the heart, especially under the acidotic conditions associated with ischemia. Like ENT1 inhibitors, ENT4 inhibitors should work specifically on ischemic tissues. Theoretically, ENT4 inhibitors do not affect tissues that rely on ENT1 for de novo nucleotide synthesis. They also have no interaction with anticancer and antiviral nucleosides. Development of specific ENT4 inhibitors may open a new avenue in research on ischemic heart disease therapy.

Key Words: nucleoside transporters, adenosine, cardioprotection, ischemia

(*J Cardiovasc Pharmacol*™ 2015;65:517–521)

Received for publication September 7, 2014; accepted November 14, 2014.

From the *Ethnic Drug Screening & Pharmacology Center, Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming, China; and †Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China.

Supported by the Natural Science Foundation of China (No. 81160404, Principal Investigator: C. Yang).

The authors report no conflicts of interest.

Reprints: George P. H. Leung, PhD, Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China (e-mail: gphleung@hkucc.hku.hk).

INTRODUCTION

Ischemic heart disease is a major cause of heart failure and mortality. According to the Global Atlas on Cardiovascular Disease Prevention and Control published by the World Health Organization in 2011, an estimated 17.3 million people died of cardiovascular diseases in 2008, representing 30% of all global deaths. About 40% of these deaths were due to ischemic heart disease. Reperfusion therapies, such as percutaneous transluminal coronary angioplasty, coronary stenting, and thrombolytic therapy, are the first-line treatments for ischemic heart disease because immediate restoration of blood flow to ischemic myocardium can limit infarct size and reduce mortality. Unfortunately, the reperfusion itself paradoxically induces myocardial injury (a phenomenon known as reperfusion injury), which attenuates the benefits of myocardial reperfusion.[1] In view of this, a great deal of research has been performed to search for pharmacological agents that can render cardiomyocytes more resistant to the deleterious effects of ischemia–reperfusion injury.

Adenosine is an endogenous purine nucleoside that plays a crucial role in modulating various physiological functions in the cardiovascular system. Adenosine levels in blood and interstitial fluid increase in response to cell injury and stress, for instance during hypoxia and ischemia. This is because a large amount of adenosine is produced from the breakdown of adenine nucleotides by ecto-5′-nucleotidase. The adenosine released during preconditioning by short periods of ischemia followed by reperfusion can induce cardioprotection for subsequent sustained ischemia.[2,3] This effect is mediated through the activation of A_1 and A_3 adenosine receptors in cardiomyocytes and involves protein kinase C and mitochondrial K_{ATP} channels.[4] The increased extracellular level of adenosine also causes vasodilation, by acting through A_2 adenosine receptors on vascular smooth muscle cells, resulting in increased blood flow to and oxygenation of ischemic tissues.[5] In addition to cardioprotective and vasodilatory effects, adenosine reduces vascular smooth muscle cell proliferation,[6] inhibits platelet aggregation,[7] and attenuates the inflammatory response.[8] Therefore, it has been suggested that adenosine may slow down the vascular remodeling process observed in hypertension and atherosclerosis.

Adenosine is currently used as an antiarrhythmic drug for the treatment of supraventricular tachycardia. Adenosine infusion can also reduce infarct size significantly.[9,10] However, the therapeutic applications of adenosine in ischemic diseases are limited by its short biological half-life, which is less than 30 seconds. This is due to the rapid uptake of extracellular

adenosine into cells by nucleoside transporters and the subsequent metabolism of adenosine into inosine and adenosine monophosphate by adenosine deaminase and adenosine kinase, respectively.[11,12] The problem of the short half-life can be overcome by the use of adenosine receptor agonists. However, like adenosine, these produce systemic side effects such as hypotension, renal diuresis, bradycardia, and sedation.[13,14]

NUCLEOSIDE TRANSPORTERS IN THE CARDIOVASCULAR SYSTEM

There are 2 major classes of nucleoside transporter in mammalian cells. The equilibrative nucleoside transporters (ENTs) are facilitated diffusion systems and are sodium independent. Four types of ENT have been characterized, among which ENT1 and ENT2 are the most widely studied. They are plasma membrane proteins that are broadly selective for purine and pyrimidine nucleosides.[15] They can be distinguished from each other by their sensitivity to inhibition by nitrobenzylmercaptopurine riboside (NBMPR). ENT1 is inhibited by nanomolar concentrations of NBMPR, whereas ENT2 is resistant to NBMPR at up to 1 μM.[16] Both ENT1 and ENT2 can transport nucleobases such as hypoxanthine, adenine, guanine, uracil, and thymine, but the efficiency and apparent affinity with which ENT1 transports nucleobases are lower than those for ENT2.[17–19] ENT3 is a membrane transporter associated with intracellular organelles such as lysosomes.[20] It can transport both purine and pyrimidine nucleosides. ENT4 was first characterized as a low-affinity high-capacity transporter for monoamines, rather than a nucleoside transporter.[21] The ability of ENT4 to transport nucleosides was confirmed in 2006.[22] Unlike other ENT subtypes, ENT4 is not broadly specific for nucleosides but mainly transports adenosine. Interestingly, the activity of ENT4 is low at neutral pH but is greatly increased at acidic pH.[22]

Another major class of nucleoside transporter is the concentrative nucleoside transporters (CNTs). CNT-1 is pyrimidine selective, CNT-2 is purine selective, and CNT-3 is broadly selective. They are sodium-dependent systems that can transport nucleosides against a concentration gradient.[23]

Different nucleoside transporters are expressed in different tissues. In general, ENTs are ubiquitous, but CNTs are mainly found in epithelial tissues. Over 95% of adenosine uptake by human aortic smooth muscle cells is mediated by ENT1, and the rest is mediated by ENT2.[24] Adenosine transport in human umbilical vein endothelial cells is completely dependent on ENT1.[25] However, in rat microvascular endothelial cells, half of the adenosine uptake is mediated by ENT1 and half by ENT2, whereas less than 1% of adenosine uptake in these cells is attributed to CNT-2.[26] Nucleoside transporters in the heart have only been studied in rodents, not in humans. Over 90% of the adenosine transport in isolated rat cardiomyocytes was inhibited by 10 μM of NBMPR.[27] Nonetheless, the relative contributions of ENT1 and ENT2 in rat heart are still not clearly defined because 10 μM of NBMPR can completely inhibit ENT1 and a large portion of rat ENT2 activity. In HL-1 cells, a mouse cardiomyocyte cell line, 85% of the adenosine transport is mediated by ENT1, while 15% is mediated by ENT2.[28] In H9c2 cells, a rat cardiomyoblast, ENT2 was found to contribute 94% of the adenosine uptake.[29] The exact reason for such an exceptionally high expression of ENT2 in H9c2 cells is unknown. However, after H9c2 cardiomyoblasts have differentiated into cardiomyocytes, the contribution of ENT1 is significantly increased, indicating that the expression of different ENTs may be cell stage–dependent.

REGULATION OF NUCLEOSIDE TRANSPORTERS IN THE CARDIOVASCULAR SYSTEM DURING ISCHEMIA

There are only 2 studies related to the effects of ischemia on the expression and activities of nucleoside transporters. An in vitro model showed that ischemia–reperfusion has no effect on the functions of ENT1 in microvascular endothelial cells.[30] However, repression of ENT1 and ENT2 transcript and protein levels by ischemia–reperfusion was reported in liver.[31] The effects of hypoxia, which is the consequence of ischemia, on nucleoside transporters in the cardiovascular system are more widely reported. Hypoxia induces inhibition of ENT1-mediated adenosine transport in human umbilical vein endothelial cells.[32] After a short period of hypoxia (1–3 hours), a reduction in ENT1 activity results from the activation of the p42/44 kinase–dependent pathway, but after a long period of hypoxia (24 hours), this kinase is not involved.[32] Hypoxia-inducible factor 1 also plays a critical role in the downregulation of nucleoside transporters in endothelia.[33] It is supposed that hypoxia-inducible factor 1 interacts with the hypoxia-responsive element within the ENT1 promotor, thereby inhibiting expression of the gene encoding ENT1 and causing transcriptional downregulation of ENT1.[33,34] Chronic hypoxia also downregulates ENT1 expression and activity in the cardiomyocyte cell line HL-1; the mechanism may involve protein kinase Cε.[35]

Diabetic patients suffer greater morbidity from ischemia.[36] Our previous studies have shown that ENT1 expression and activity in cultured human aortic smooth muscle cells are increased by >30% if the cells are incubated in high glucose medium (which mimics the hyperglycemia of diabetes).[24] The increased expression of ENT1 involves the activation of the mitogen-activated protein kinase–dependent pathway. Consistently, ENT1-mediated adenosine transport in freshly isolated human umbilical cord arterial smooth muscle cells from pregnant diabetic patients was 3-fold higher than that in normal arterial smooth muscle cells.[37] We speculate that the increased level of ENT1 in vascular smooth muscle cells may decrease the availability of adenosine to adenosine receptors, thereby weakening the vasodilatory effect of adenosine during ischemia.

Diabetes-induced changes in ENT1 expression are tissue-specific. Expression of ENT1 is decreased in endothelial cells isolated from the umbilical veins of human diabetic subjects, in contrast to vascular smooth muscle cells.[37,38] In addition, ENT1 expression in the hearts of rats with streptozotocin-induced diabetes is lower than that in normal rats.[39] One possible explanation is that the regulation of ENT1 expression in various tissues is controlled by different signaling pathways. The reduced expression of ENT1 in the heart may also be a compensatory mechanism in response to the attenuated vasodilatory effect of adenosine in diabetes. Further experiments are required to study

whether or not hyperglycemia per se has any direct effect on isolated cardiomyocytes.

Hypertension is one of the major risk factors for ischemic heart disease, and appropriate control of blood pressure is the cornerstone of the prevention of ischemic heart disease. So far, there has been only 1 report about the association between hypertension and nucleoside transporters. The expression of ENT1 is higher in the kidneys but lower in the platelets of hypertensive rats.[40] Although there was no difference in ENT1 expression between the hearts of hypertensive and normotensive rats, an age-dependent decrease of ENT1 was observed in the hearts of hypertensive rats.[40] A preliminary study performed by the authors of this review showed that ENT1 and ENT2 are unaffected, but the expression of CNT-2 in the basilar arteries of hypertensive rats is higher than that in normotensive rats.[41] It will be of interest to study whether the upregulation of CNT-2 is a primary or secondary event in the development of hypertension.

ROLES OF ENT1 IN CARDIOPROTECTION

The involvement of ENT1 in cardioprotection is revealed by 2 findings. First, ENT1-null mice show less myocardial infarction than wild-type littermates.[42] Second, pharmacological inhibition of ENT1 and ENT2 by NBMPR and dipyridamole reduces the incidence of ventricular fibrillation and attenuates regional contractile dysfunction mediated by myocardial infarction due to reperfusion injury.[43–46] Genetic knockout or pharmacological inhibition of ENT1 is supposed to potentiate the effect of endogenous adenosine by reducing and retarding the disappearance of adenosine. In addition, inhibition of ENTs may also cause retention/trapping of adenosine within cardiomyocytes, which helps replenish essential adenine nucleotides (eg, ATP).[47]

ENT1 inhibitors should be more effective than adenosine and adenosine receptor agonists in the treatment and prevention of ischemic heart disease. Theoretically, ENT1 inhibitors have fewer systemic side effects because endogenous adenosine levels are selectively elevated in ischemic tissues undergoing accelerated adenosine formation. However, some potential drawbacks of ENT1 inhibitors may be underestimated. Apart from adenosine transport, ENT1 is responsible for the cellular uptake of other nucleosides that are critical for salvage pathways of nucleotide biosynthesis.[48,49] Therefore, inhibition of ENT1 may adversely affect tissues that lack or have low capacity for de novo nucleotide biosynthesis (eg, erythrocytes, leukocytes, intestinal mucosa, and certain brain cells).[50,51] In addition, ENT1 transports anticancer and antiviral nucleoside drugs into tumor cells and infected cells,[52] and therefore the use of ENT1 inhibitors may reduce the therapeutic effect of chemotherapy.[53–56] ENT2 inhibitors have the same problems as ENT1 inhibitors, so they are not a suitable alternative.

INHIBITION OF ENT4: A POTENTIAL APPROACH FOR CARDIOPROTECTION?

Unlike other ENT subtypes, which are broadly selective for purine and pyrimidine nucleosides, ENT4 behaves more like a monoamine transporter.[21] In the brain, ENT4 serves as a low-affinity but high-capacity transporter of neurotransmitters such as serotonin and dopamine.[57] Like ENT1 and ENT2, ENT4 is abundant in the heart, particularly in ventricular myocytes and microvascular endothelial cells.[22] However, the function of ENT4 in the heart is not known. Its ability to transport serotonin may be of physiological significance because serotonin is important in regulating cardiac development.[58] Although ENT4 transports adenosine with lower efficiency than monoamine substrates, it shows a much greater activity at acidic pH, and optimal activity occurs at approximately pH 6.0.[22] This pH dependency of ENT4 may be highly relevant to tissue ischemia because myocardial ischemia can lead to a rapid fall in interstitial fluid pH, to values as low as pH 6.6.[59] No investigation has yet been attempted to test this hypothesis.

Mice with targeted deletion of the ENT4 gene have been studied.[60] ENT4-null mice are viable and fertile, with no overt physiological abnormalities. Blood chemistry shows that serum biomarkers in wild-type and ENT4-null mice are basically the same. The lack of an overt phenotype in ENT4-null mice indicates that ENT4 is not essential physiologically under normal conditions. However, monoamine uptake is significantly reduced in the choroid plexus of ENT4-null mice. Further experiments are necessary to study whether the adenosine uptake in cardiomyocytes of ENT4-null mice is impaired or whether the heart of ENT4-null mice is more resistant to ischemic injury.

Like ENT1 inhibitors, it is expected that ENT4 inhibitors will prolong and amplify the effects of endogenous adenosine generated in ischemic tissues. However, ENT4 inhibitors may be superior to ENT1 inhibitors in 2 aspects. First, ENT4 is specific for adenosine,[21,22] so its inhibitors should have fewer adverse effects on tissues that lack or have low capacity for de novo nucleotide biosynthesis. Second, anticancer and antiviral nucleoside drugs are not substrates of ENT4[21]; they do not rely on ENT4 for their transportation into target cells. Therefore, the use of ENT4 inhibitors should not affect the efficacy of these nucleoside drugs. ENT4 is inhibited by decynium-22, GBP12935, and citalopram. Unfortunately, these compounds are not potent, not specific for ENT4, and produce unwanted adverse reactions.[61,62] Wang et al[63] have recently studied a series of dipyridamole analogs and found that an analog with a 2,6-di (N-monohydroxyethyl) substitution on the pyrimidopyrimidine ring can inhibit ENT4 with an IC_{50} of 74.4 nM, which is about 38 times more potent than dipyridamole. The selectivity of this compound is about 80-fold and 20-fold relative to ENT1 and ENT2, respectively. This compound may be a useful pharmacological tool and potential lead for ENT4-based therapeutics, especially in ischemic heart diseases. A potential drawback of using ENT4 inhibitors is that they may increase the levels of catecholamine (eg, dopamine, norepinephrine, and epinephrine) in tissues. In addition to the vasoconstrictor effects, the abundance of tissue catecholamines may make monoamine oxidase–derived hydrogen peroxide production more prominent.[64] This may have harmful effect during ischemia–reperfusion injury, and this problem should be addressed in future research.

CONCLUSIONS

ENT4 plays a significant role in adenosine transport in cardiomyocytes, especially in ischemic conditions. Inhibition

of ENT4 may be an attractive approach in the treatment of ischemic heart disease. There are several potential advantages. First, ENT4 inhibitors work specifically in ischemic areas, so there should be few adverse systemic side effects. Second, ENT4 inhibitors should not affect tissues that lack or have low capacity for de novo nucleotide biosynthesis. Third, ENT4 inhibitors should have no interaction with anticancer and antiviral nucleoside drugs. Further studies are required to reveal the physiological roles of ENT4 in the heart. Research on pharmacological inhibitors of ENT4 has been started, and hopefully a novel cardiovascular protective drug can be developed.

REFERENCES

1. Kalogeris T, Baines CP, Krenz M, et al. Cell biology of ischemia/reperfusion injury. *Int Rev Cell Mol Biol.* 2012;298:229–317.
2. Donato M, Gelpi RJ. Adenosine and cardioprotection during reperfusion–an overview. *Mol Cell Biochem.* 2003;251:153–159.
3. Headrick JP, Hack B, Ashton KJ. Acute adenosinergic cardioprotection in ischemic-reperfused hearts. *Am J Physiol Heart Circ Physiol.* 2003; 285:H1797–H1818.
4. Mubagwa K, Flameng W. Adenosine, adenosine receptors and myocardial protection: an updated overview. *Cardiovasc Res.* 2001;52:25–39.
5. Collis MG. The vasodilator role of adenosine. *J Pharm Pharmacol.* 2003;55:519–526.
6. Dubey RK, Gillespie DG, Mi Z, et al. Smooth muscle cell-derived adenosine inhibits cell growth. *Hypertension*. 1996;27:766–773.
7. Picano E, Abbracchio MP. European stroke prevention study-2 results: serendipitous demonstration of neuroprotection induced by endogenous adenosine accumulation? *Trends Pharmacol Sci.* 1998;19:14–16.
8. Sands WA, Palmer TM. Adenosine receptors and the control of endothelial cell function in inflammatory disease. *Immunol Lett.* 2005;101:1–11.
9. Kloner RA, Forman MB, Gibbons RJ, et al. Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. *Eur Heart J.* 2006;27:2400–2405.
10. Ross AM, Gibbons RJ, Stone GW, et al; MISTAD-II Investigators. A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *J Am Coll Cardiol.* 2005;45:1775–1780.
11. Dawicki DD, Agarwal KC, Parks RE Jr. Potentiation of the antiplatelet action of adenosine in whole blood by dipyridamole or dilazep and the cAMP phosphodiesterase inhibitor, RA 233. *Thromb Res.* 1986;43: 161–175.
12. Yeung PK, Mosher SJ, Macrae DA, et al. Effect of diltiazem and its metabolites on the uptake of adenosine in blood: an invitro investigation. *J Pharm Pharmacol.* 1991;43:685–689.
13. Jacobson KA, van Galen PJ, Williams M. Adenosine receptors: pharmacology, structure–activity relationships, and therapeutic potential. *J Med Chem.* 1992;35:407–422.
14. Williams M. Purines: from premise to promise. *J Auton Nerv Syst.* 2000; 81:285–288.
15. Baldwin SA, Beal PR, Yao SY, et al. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch.* 2004;447:735–743.
16. Ward JL, Sherali A, Mo ZP, et al. Kinetic and pharmacological properties of cloned human equilibrative nucleoside transporters, ENT1 and ENT2, stably expressed in nucleoside transporter-deficient PK15 cells. ENT2 exhibits a low affinity for guanosine and cytidine but a high affinity for inosine. *J Biol Chem.* 2000;275:8375–8381.
17. Robillard KR, Bone DB, Hammond JR. Hypoxanthine uptake and release by equilibrative nucleoside transporter 2 (ENT2) of rat microvascular endothelial cells. *Microvasc Res.* 2008;75:351–357.
18. Yao SY, Ng AM, Cass CE, et al. Nucleobase transport by human equilibrative nucleoside transporter 1 (hENT1). *J Biol Chem.* 2011;286: 32552–32562.
19. Yao SY, Ng AM, Vickers MF, et al. Functional and molecular characterization of nucleobase transport by recombinant human and rat equilibrative nucleoside transporters 1 and 2. Chimeric constructs reveal a role for the ENT2 helix 5-6 region in nucleobase translocation. *J Biol Chem.* 2002;277:24938–24948.
20. Baldwin SA, Yao SY, Hyde RJ, et al. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. *J Biol Chem.* 2005;280: 15880–15887.
21. Engel K, Zhou M, Wang J. Identification and characterization of a novel monoamine transporter in the human brain. *J Biol Chem.* 2004;279: 50042–50049.
22. Barnes K, Dobrzynski H, Foppolo S, et al. Distribution and functional characterization of equilibrative nucleoside transporter-4, a novel cardiac adenosine transporter activated at acidic pH. *Circ Res.* 2006;99:510–519.
23. Gray JH, Owen RP, Giacomini KM. The concentrative nucleoside transporter family, SLC28. *Pflugers Arch.* 2004;447:728–734.
24. Leung GP, Man RY, Tse CM. D-Glucose upregulates adenosine transport in cultured human aortic smooth muscle cells. *Am J Physiol Heart Circ Physiol.* 2005;288:H2756–H2762.
25. Sobrevia L, Jarvis SM, Yudilevich DL. Adenosine transport in cultured human umbilical vein endothelial cells is reduced in diabetes. *Am J Physiol.* 1994;267:C39–C47.
26. Archer RG, Pitelka V, Hammond JR. Nucleoside transporter subtype expression and function in rat skeletal muscle microvascular endothelial cells. *Br J Pharmacol.* 2004;143:202–214.
27. Ford DA, Rovetto MJ. Rat cardiac myocyte adenosine transport and metabolism. *Am J Physiol.* 1987;252:H54–H63.
28. Chaudary N, Shuralyova I, Liron T, et al. Transport characteristics of HL-1 cells: a new model for the study of adenosine physiology in cardiomyocytes. *Biochem Cell Biol.* 2002;80:655–665.
29. Leung GP, Tse CM, Man RY. Characterization of adenosine transport in H9c2 cardiomyoblasts. *Int J Cardiol.* 2007;116:186–193.
30. Bone DB, Antic M, Vilas G, et al. Oxidative stress modulates nucleobase transport in microvascular endothelial cells. *Microvasc Res.* 2014;95:68–75.
31. Zimmerman MA, Tak E, Ehrentraut SF, et al. Equilibrative nucleoside transporter (ENT)-1-dependent elevation of extracellular adenosine protects the liver during ischemia and reperfusion. *Hepatology*. 2013;58:1766–1778.
32. Casanello P, Torres A, Sanhueza F, et al. Equilibrative nucleoside transporter 1 expression is downregulated by hypoxia in human umbilical vein endothelium. *Circ Res.* 2005;97:16–24.
33. Eltzschig HK, Abdulla P, Hoffman E, et al. HIF-1-dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J Exp Med.* 2005;202:1493–1505.
34. Löffler M, Morote-Garcia JC, Eltzschig SA, et al. Physiological roles of vascular nucleoside transporters. *Arterioscler Thromb Vasc Biol.* 2007; 27:1004–1013.
35. Chaudary N, Naydenova Z, Shuralyova I, et al. Hypoxia regulates the adenosine transporter, mENT1, in the murine cardiomyocyte cell line, HL-1. *Cardiovasc Res.* 2004;61:780–788.
36. Blendea MC, McFarlane SI, Isenovic ER, et al. Heart disease in diabetic patients. *Curr Diab Rep.* 2003;3:223–229.
37. Aguayo C, Sobrevia L. Nitric oxide, cGMP and cAMP modulate nitrobenzylthioinosine-sensitive adenosine transport in human umbilical artery smooth muscle cells from subjects with gestational diabetes. *Exp Physiol.* 2000;85:399–409.
38. Parodi J, Flores C, Aguayo C, et al. Inhibition of nitrobenzylthioinosine-sensitive adenosine transport by elevated D-glucose involves activation of P2Y2 purinoceptors in human umbilical vein endothelial cells. *Circ Res.* 2002;90:570–577.
39. Pawelczyk T, Podgorska M, Sakowicz M. The effect of insulin on expression level of nucleoside transporters in diabetic rats. *Mol Pharmacol.* 2003;63:81–88.
40. Williams EF, Udofia UP, Brooks GR, et al. Changes in renal, platelet and cardiac nitrobenzylthioinosine binding in spontaneously hypertensive rats. *Life Sci.* 1990;46:1007–1012.
41. Leung GP, Ho EY, Li RW, et al. Diabetes- and hypertension-induced upregulation of concentrative nucleoside transporter-2 in endothelial cells. *J Vasc Res.* 2009;46:178.
42. Rose JB, Naydenova Z, Bang A, et al. Equilibrative nucleoside transporter 1 plays an essential role in cardioprotection. *Am J Physiol Heart Circ Physiol.* 2010;298:H771–H777.
43. Abd-Elfattah AS, Jessen ME, Lekven J, et al. Differential cardioprotection with selective inhibitors of adenosine metabolism and transport: role of purine release in ischemic and reperfusion injury. *Mol Cell Biochem.* 1998;180:179–191.

44. Figueredo VM, Diamond I, Zhou HZ, et al. Chronic dipyridamole therapy produces sustained protection against cardiac ischemia-reperfusion injury. *Am J Physiol.* 1999;277:H2091–H2097.
45. Riksen NP, Oyen WJ, Ramakers BP, et al. Oral therapy with dipyridamole limits ischemia-reperfusion injury in humans. *Clin Pharmacol Ther.* 2005;78:52–59.
46. Zhu Z, Hofmann PA, Buolamwini JK. Cardioprotective effects of novel tetrahydroisoquinoline analogs of nitrobenzylmercaptopurine riboside in an isolated perfused rat heart model of acute myocardial infarction. *Am J Physiol Heart Circ Physiol.* 2007;292:H2921–H2926.
47. Abd-Elfattah AS, Jessen ME, Wechsler AS. Nucleoside trapping during reperfusion prevents ventricular dysfunction, "stunning," in absence of adenosine. Possible separation between ischemic and reperfusion injury. *J Thorac Cardiovasc Surg.* 1994;108:269–278.
48. Fontenelle LJ, Henderson JF. An enzymatic basis for the inability of erythrocytes to synthesize purine ribonucleotides de novo. *Biochim Biophys Acta.* 1969;177:175–176.
49. Mackinnon AM, Deller DJ. Purine nucleotide biosynthesis in gastrointestinal mucosa. *Biochim Biophys Acta.* 1973;319:1–4.
50. Weber G, Lui MS, Natsumeda Y, et al. Salvage capacity of hepatoma 3924A and action of dipyridamole. *Adv Enzyme Regul.* 1983;21:53–69.
51. Betageri GV, Szebeni J, Hung K, et al. Effect of dipyridamole on transport and phosphorylation of thymidine and 3'-azido-3'-deoxythymidine in human monocyte/macrophages. *Biochem Pharmacol.* 1990;40:867–870.
52. Leung GP, Tse CM. The role of mitochondrial and plasma membrane nucleoside transporters in drug toxicity. *Expert Opin Drug Metab Toxicol.* 2007;3:705–718.
53. Ibarra KD, Pfeiffer JK. Reduced ribavirin antiviral efficacy via nucleoside transporter-mediated drug resistance. *J Virol.* 2009;83:4538–4547.
54. Iikura M, Furihata T, Mizuguchi M, et al. ENT1, a ribavirin transporter, plays a pivotal role in antiviral efficacy of ribavirin in a hepatitis C virus replication cell system. *Antimicrob Agents Chemother.* 2012;56:1407–1413.
55. Kubota M, Takimoto T, Kitoh T, et al. Prevention of 1-beta-D arabinofuranosylcytosine toxicity by 4-nitrobenzyl-6-thioinosine or dipyridamole in human leukemia cell lines. *Anticancer Res.* 1988;8:339–342.
56. Takaai M, Morishita H, Ishida K, et al. Contribution of Na+-independent nucleoside transport to ribavirin uptake in the rat intestine and human epithelial LS180 cells. *Eur J Pharmacol.* 2008:601:61–65.
57. Duan H, Wang J. Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. *J Pharmacol Exp Ther.* 2010;335:743–753.
58. Nebigil CG, Maroteaux L. A novel role for serotonin in heart. *Trends Cardiovasc Med.* 2001;11:329–335.
59. Carrier M, Trudelle S, Thai P, et al. Ischemic threshold during cold blood cardioplegic arrest: monitoring with tissue pH and pO2. *J Cardiovasc Surg (Torino).* 1998;39:593–597.
60. Duan H, Wang J. Impaired monoamine and organic cation uptake in choroid plexus in mice with targeted disruption of the plasma membrane monoamine transporter (Slc29a4) gene. *J Biol Chem.* 2013;288:3535–3544.
61. Russ H, Friedgen B, Kőnigs B, et al. Pharmacokinetic and alpha 1-adrenoceptor antagonistic properties of two cyanine-type inhibitors of extraneuronal monoamine transport. *Naunyn Schmiedebergs Arch Pharmacol.* 1996;354:268–274.
62. Tella SR, Goldberg SR. Monoamine transporter and sodium channel mechanisms in the rapid pressor response to cocaine. *Pharmacol Biochem Behav.* 1998;59:305–312.
63. Wang C, Lin W, Playa H, et al. Dipyridamole analogs as pharmacological inhibitors of equilibrative nucleoside transporters. Identification of novel potent and selective inhibitors of the adenosine transporter function of human equilibrative nucleoside transporter 4 (hENT4). *Biochem Pharmacol.* 2013;86:1531–1540.
64. Kaludercic N, Carpi A, Menabò R, et al. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. *Biochim Biophys Acta.* 2011;1813:1323–1332.